Diversity of bacterial endosymbionts in siphonous and siphonocladalean green algae

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INTRODUCTION

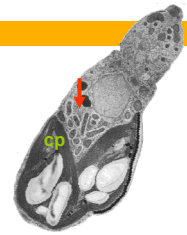
Many eukaryotes maintain close physiological associations with bacteria that reside within or on the surface of the cells.

Microbial symbionts have been well documented in various animals, land plants and heterotrophic protists.

Bacteria are also commonly found in close associations with algae. These bacteria can be facultative or obligate, ecto- or endosymbionts.

Epiphytic bacteria have been found to be ubiquitous on the external surfaces of marine macrophytes.

Many algae, including the siphonous green seaweeds *Bryopsis* and *Caulerpa*, naturally harbour endosymbiotic bacteria. →



TEM-photo of female gamete of *Bryopsis hypnoides* with a cluster of bacteria (arrows) on top of the chloroplast (cp) (Burr & West 1970)

This study focuses on the identity and diversity of endosymbiotic bacteria in the marine siphonous and siphonocladalean green algae ***Bryopsis*** and ***Boergesenia***.

METHODOLOGY

Photo: Heroen Verbruggen

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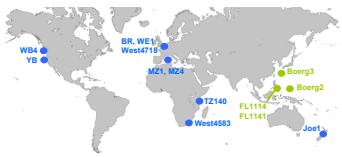
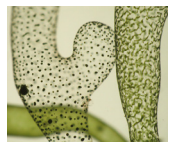
Sampling ● *Bryopsis* ● *Boergesenia*



Isolation of endophytic bacteria

Bryopsis:
Peri- and epiphytes removed chemically and enzymatically

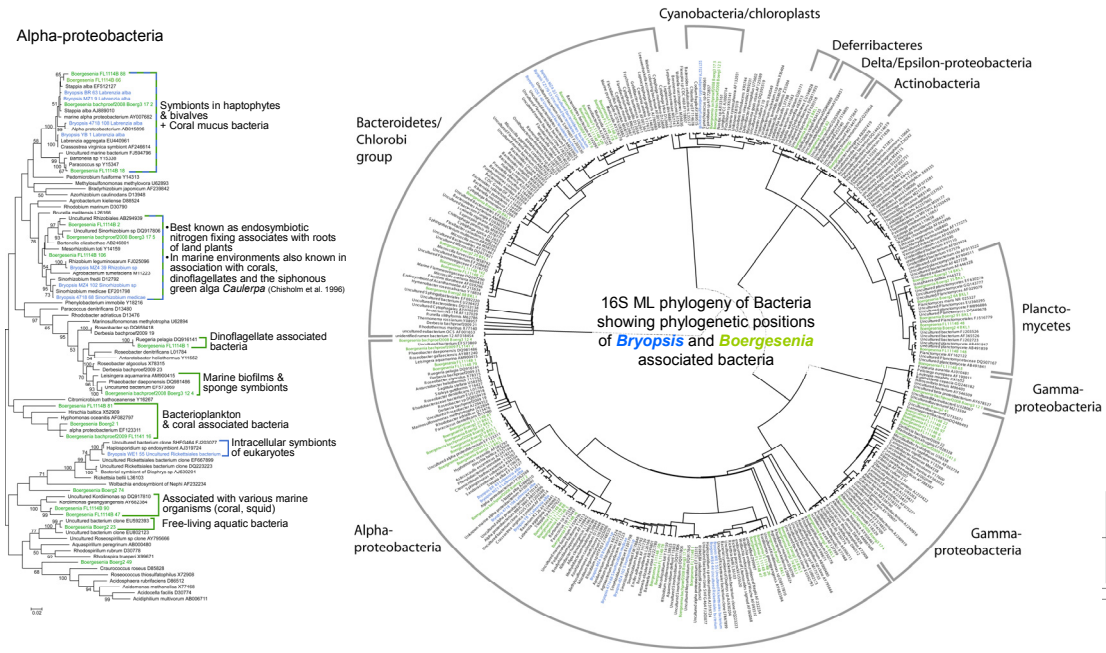
Boergesenia:
Isolation of segregative cells →



Molecular techniques

Total DNA extraction (CTAB) → 16S rDNA PCR with universal primers → Cloning of PCR products with pGEM-T vector system → Screening of clones using DGGE and RFLP → DNA sequencing → initial BLAST identification of 16S sequences → Maximum likelihood phylogenetic analyses

RESULTS AND CONCLUSIONS



- *Bryopsis* appears to be associated with only a limited number of bacteria, belonged to one Bacteroidetes and three Alpha-proteobacteria clades: Rhizobiales, *Labrenzia* and Rickettsiales.
- *Boergesenia* is characterized by more complex communities of microbial endophytes: bacteria are phylogenetically divers, spanning 8 main bacterial groups.
- The occurrence of distinct bacterial clades with well-known symbiotic features (e.g. Rhizobiales, Rickettsiales, *Labranzia*) in different isolates of *Bryopsis* and *Boergesenia* suggests an obligate and long term-term maintenance of these bacterial microbiota. On the other hand, the presence of a wide diversity of intracellular bacteria in *Boergesenia*, including known symbionts as well as free-living bacteria could indicate that part of the endophytic bacteria in *Boergesenia* have been repeatedly acquired from the marine environment.
- Future research will focus on visualization of the endosymbionts with fluorescent probes to confirm their identification and location inside the algal host. Different taxa of siphonous and siphonocladalean green algae will be sampled in order to better understand the evolution of these symbiotic microbial communities.

References

Burr F & West JA (1970) Light and electron microscope observations on the vegetative and reproductive structures of *Bryopsis hypnoides*. Phycologia 9: 17-37.

Chisholm JRM et al. (1996) Roots in mixotrophic algae. Nature 381: 382.

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